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Modulatory effect of inulin with soya isoflavones on plasma lipid profile and liver SCD-18 index in rats with induced type-2 diabetes mellitus

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Summary. Obesity and type-2 diabetes are often associated with nonalcoholic fatty liver disease (NAFLD). Soya isoflavones act as antidiabetic agents and protect against NAFLD. There are data suggesting that inulin may increase the plasma concentration and effect of soya isoflavones. The aim of the present study was to compare the effect of soya isoflavones, as opposed to the effect of soya isoflavones with inulin, on plasma lipid profile, liver morphology, and liver fatty acids in rats with induced type-2 diabetes mellitus.

Data were collected on thirty-six male Sprague-Dawley rats divided into control and diabetic groups. Animals in the diabetic (DM) group were on a high-fat diet and were injected with low doses of streptozotocin. Animals in the control groups were fed a regular diet and were injected with a buffer. After the injections, the animals were divided into three groups of nondiabetic rats (nDM)-controls (c-nDM), rats treated with isoflavones (IS-nDM), and rats treated with isoflavones plus inulin (IS+IN-nDM)-and three parallel diabetic (DM) subgroups: controls (c-DM), rats treated with isoflavone (IS-DM), and rats treated with isoflavones plus inulin (IS+IN-DM). Hepatic steatosis and fibrosis were examined using hematoxylin-eosin staining and Mallory's trichrome methods respectively. Liver fatty acids were extracted and analyzed by gas chromatography. A lipid blood test was performed.

The study showed significant changes in liver fatty acids, liver morphology, and plasma lipid profile. The estimated SCD-18 index significantly decreased in both the control and DM groups after isoflavone supplementation. The level of liver steatosis and fibrosis also decreased after isoflavone supplementation in the DM groups. The plasma lipid profile showed increased levels of HDL-C after isoflavone supplementation in the DM groups.

These results support the protective use of isoflavones in liver steatosis and as beneficial to plasma lipid profile in individuals with diabetes. A novelty of this work is its comparison of supplementation using soya isoflavones with supplementation using both soya isoflavones and inulin. Surprisingly, additional supplementation with inulin modulates the positive effect of isoflavones.

Key words: Diabetes, Isoflavones, Inulin, Fatty acids, Liver

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Introduction

Obesity and type-2 diabetes (DMT2) are often associated with nonalcoholic fatty liver disease (NAFLD) (Firneish, 2014). NAFLD is a condition in which lipids accumulate in the cytoplasm of hepatocytes (steatosis), which develops into

steatohepatitis, associated with progressive inflammation of the liver; this ultimately leads to cirrhosis (hepatic fibrosis) (El-Sayyad et al., 2014). NAFLD development is related to genetic and environmental factors, such as insufficient secretion of insulin by β cells, together with obesity caused by increased calorie intake and decreased physical activity (Birkenfeld and Shulman, 2014; Harikrashna et al., 2015). The abnormal secretion of insulin leads to hyperglycemia, which is associated with impaired low density lipoprotein secretion, increased triglyceride synthesis, abnormal hepatic fatty acid oxidation, and hepatic lipid accumulation (El-Sayyad and El-Shahary, 2016). Patients with NAFLD thus have many clinical characteristics, such as disordered glucose metabolism, abdominal obesity, elevated triglyceride levels, low levels of high-density lipoprotein cholesterol, and elevated blood pressure. Elevated levels of circulating free fatty acids, due to their release from adipose tissue and the *de novo* synthesis of fatty acids, result in increased delivery of fatty acids to the liver, which then begins to synthesis excess triglycerides. Synthesis of triglycerides is further increased by the impaired oxidation of hepatic fatty acid, which is due to insulin resistance (Harikrashna et al., 2015).

Numerous studies have revealed that diets rich in soya isoflavones help normalize the level of cholesterol, body mass index, and volume of adipose tissue. Soya isoflavones act as antidiabetic agents and protect against NAFLD (Gilbert and Liu, 2013; Qui and Chen, 2015). Soya is rich in isoflavones that do not accumulate in tissues and which also act as antioxidants (Tyung et al., 2010). Agents with antioxidative properties are highly recommended to patients with type-2 diabetes, because prolonged hyperglycemia leads to oxidative stress, which may be one of the reasons for the death of insulin-producing cells.

The use of soya isoflavones to protect against NAFLD is well documented, so it is worth finding a means of enhancing the positive effects of these substances. There are findings suggesting that the concentration of plasma soya isoflavones increases with additional supplementation with inulin (Piazza et al., 2007). Inulin is a natural nondigestible food ingredient that may stimulate the growth or metabolic activity of certain bacteria (such as lactobacilli, bacteroides, and bifidobacteria) in the human colon (McFarlane and Cumming, 1991). B-glucosidases of intestinal microflora can hydrolyze isoflavones to aglycones and promote their absorption. Moreover, inulin, which is a type of dietary fiber, is fermented in the digestive tract, resulting in the formation of short chain fatty acids which have the ability to lower serum glucose and lipid levels in the body itself (Bonsu and Johnson, 2012).

The aim of the present study was to compare the effect of soya isoflavones alone and soya isoflavones with inulin on plasma lipid profile, liver morphology, and liver fatty acids in rats with induced type-2 diabetes mellitus.

Materials and methods

Animal care and maintenance

Thirty-six male Sprague-Dawley (SD) rats were obtained from Animalab (Germany) at the age of 9-10 weeks. The animals were maintained in a temperature-controlled environment under standard conditions of lighting (12h:12h light-dark cycle). All animals were allowed to acclimatize for a week to the normal diet before grouping. After a one week accommodation period, the rats were randomly divided into two main groups. Animals in the control group (C; n=18 rats) were fed a regular diet, while rats in the high-fat diet group (HFD; n=18) were fed high-fat diet to induce insulin resistance. Diet composition: the regular chow included 5% fat (rape oil), 52% carbohydrate, and 20% protein, with a total calorific value of 20 kJ/kg; the high-fat diet consisted of 20% fat (lard), 45% carbohydrate, and 22% protein, with a total calorific value of 40 kJ/kg (following Zhang et al., 2008). Animals in both groups (C, and HFD) were housed in plastic cages in groups of three and were permitted *ad libitum* consumption of water and chow till the end of the experiment. Two months later, the rats in the HFD group were treated with streptozotocin (STZ; Sigma-Aldrich) to induce type-2 diabetes (Zhang et al., 2008). Rats received two intraperitoneal injections of streptozotocin (STZ, 30 mg/kg b.w.) (Sigma Aldrich) three days apart (DM group), while the control group animals (n-DM group) were injected with 0.25 mL/kg of 0.9% NaCl. Then, three days after the first injection, blood glucose levels were tested using a blood glucose meter. Rats with glucose levels greater than 16.67 mmol/L were not injected with STZ a second time. After the injections, the animals in the nondiabetic group (nDM) were randomly divided into three subgroups (n=6)-namely, controls (c-nDM), rats treated with 100 mg/kg b.w. isoflavones (IS-nDM), and rats treated with 100 mg/kg b.w. isoflavones plus 100 mg/kg b.w. inulin (IS+IN-nDM). The STZ-treated diabetic rats (DM) were divided into three parallel subgroups (n=6)-controls (c-DM), rats treated with 100 mg/kg b.w. isoflavones (IS-DM), and rats treated with 100 mg/kg b.w. isoflavones plus 100 mg/kg b.w. inulin (IS+IN-DM). The isoflavones and inulin were administered *per* os for 30 days, once per day in the morning, in the form of small pellets of isoflavones or isoflavones plus inulin, pressed into a piece of bread. The pellet was served to each experimental rat. The animals willingly ate the pellets from the hand of the person performing the experiment. The rats in both control subgroups (c-nDM and c-DM) received pellets without the test substances. The rats were allowed to continue to consume their respective diets until the end of the experiment. At the end of experiment, the rats were six months old. After 12-14 hours of fasting, the animals were sacrificed under Morbital anesthesia (2 mL/kg b.w.). Blood was taken directly from the heart and the lipid blood test was

performed (HDL cholesterol, LDL cholesterol, total cholesterol, triglycerides). The experiment was conducted in accordance with Polish Law and with the approval of the ethics committee of the Pomeranian Medical University. The assumptions of the experiment are shown in Table 1.

Histopathological evaluation

For the histological examination, the livers were fixed in a 4% buffered formalin solution, embedded in paraffin blocks, and cut into 4-μm sections. For the morphological analysis, a series of liver slides were stained with hematoxylin-eosin (H-E) and Mallory's trichrome method (Bio-Optica, Italy) was used to analyze fibrotic changes. Hepatic steatosis and fibrosis were assessed using ten light microscopic fields (viewed on each section) and scored for the severity of hepatic steatosis and fibrosis (Xu et al., 2010). Hepatic steatosis was examined according to the criteria of Kleiner et al. (2005) and Xu et al. (2010): grade 0: no fat; grade 1: steatosis occupying less than 33% of the hepatic parenchyma; grade 2: steatosis occupying 34%-66% of the hepatic parenchyma; grade 3: steatosis occupying more than 66% of the hepatic parenchyma. To evaluate the staging of hepatic fibrosis, the following criteria were used: 0: none; 1: mild, zone 3, perisinusoidal; 2: moderate, zone 3, perisinusoidal; 3: portal/periportal; 4: bridging fibrosis (Kleiner et al., 2005; Xu et al., 2010).

Isolation of fatty acids

Total lipids were extracted using the Folch method (Folch et al., 1957). 60 mg of liver was homogenized and saponified with 3 ml of methanol:chloroform (1:2). Samples were centrifuged (1250 g at 4°C) and 1 mL of supernatant was saponified in 2 mol/l KOH methanolic solution at 70°C for 20 min, and then methylated with 2 ml of 14% boron trifluoride in methanol under the same conditions. Then 2 mL of n-hexane and 10 mL of saturated NaCl solution were added. One milliliter of the n-hexane phase was collected for analysis.

Analysis of fatty acid methyl esters

Gas chromatography was performed using an Agilent Technologies 7890A GC System (Supelcowax 10 Capillary GC Column; 15×0.10 mm, 0.10 μm). Chromatographic conditions were as follows: the initial temperature was 60°C for 0 min, increased at a rate of 40°C/min to 160°C (0 min), increased at a rate of 30°C/min to 190°C (0.5 min), and then increased at a rate of 30°C /min to 230°C for 2.6 min, where it was maintained for 4.9 min. The total analysis lasted approximately 8 min, and the gas flow rate was 0.8 mL/min, with hydrogen as the carrier gas. Fatty acids were identified by comparing their retention times with those of commercially available standards.

Fatty acid indices

Desaturase index was estimated as the ratio of liver fatty acid product to liver fatty acid precursor, using the following: SCD-16=C16:1n-7/C16:0, SCD-18=C18:1n-9/C18:0 (Nakamura and Nara, 2004).

Hepatic *de novo* lipogenic index (DNL) was estimated as the ratio of palmitic acid (C16:0) to linoleic acid (C18:2n-6), according to the following: DNL index=C16:0/C18:2n-6 (Chang et al., 2008).

Statistical analysis

Statistica 7.1 software was used for the statistical analysis, and results were expressed as means±standard deviations. As the distribution was in most cases normal (Shapiro-Wilk test), the parametric t-test was used for comparisons between groups. To compare the categorical variables, the chi-squared test was used (MedCalc Statistical Software version 16.4.3). $P<0.05$ was considered to be significant.

Results

Histological evaluation of the liver

No rat died in either group during the whole experimental period.

Hepatic steatosis and fibrosis

Histological evaluation showed no abnormalities in the liver tissues of all three groups of rats without

Table 1. Schema of the experiment.

Control group (n=18 rats)			High Fat Diet group (n=18 rats)		
INJECTIONS: 0.9% NaCl; 0.25 mL/kg b.w. Non-diabetes rats (n-DM)			INJECTIONS: STZ; 30 mg/kg b.w. Diabetes rats (DM)		
6 rats C-nDM	6 rats IS-nDM 100 mg/kg b.w.	6 rats IS+IN-nDM 100+100 mg/kg b.w.	6 rats C-DM	6 rats IS-DM 100 mg/kg b.w.	6 rats IS+IN-DM 100+100 mg/kg b.w.

C-nDM, control nondiabetic; IS-nDM, nondiabetic group supplemented with isoflavones (100 mg/kg b.w.); IS+IN-nDM, nondiabetic group supplemented with both isoflavones (100 mg/kg b.w.) and inulin (100 mg/kg b.w.); C-DM, control diabetic group; IS-DM, diabetic group supplemented with isoflavones (100 mg/kg b.w.); IS+IN-DM, diabetic group supplemented with both isoflavones (100 mg/kg b.w.) and inulin (100 mg/kg b.w.).

diabetes (Fig. 1A). Hepatocytes were arranged in typical plates of cords, and the liver parenchyma was arranged in hexagonal lobules with the portal areas at the corners and the centrally located central vein in each lobule. The livers of rats with induced diabetes showed mixed types of hepatic steatosis in all three zones of the hepatocytes in hepatic lobules. The predominant type of steatosis was the microvesicular type, in which the hepatocytes contain small lipid droplet depositions in the cytoplasm (Fig. 1D). Supplementation with soya isoflavones and with soya isoflavones plus inulin decreased the number of hepatocytes with lipid droplets (Fig. 1E,F, Table 2). Hepatic fibrosis was not observed in non-diabetic group (Table 2) (Fig. 2A) but it was present in some of the rats with induced diabetes. In the C-DM group, mild perisinusoidal fibrosis was present in four rats, while moderate perisinusoidal fibrosis was seen in one rat (Table 2) (Fig. 2B). A decreased number of animals with hepatic fibrosis was observed among the rats supplemented with either soya isoflavones alone or with both soya isoflavones and inulin (Table 2) (Fig. 2C,D).

Baseline plasma lipid profile

The baseline plasma lipid profile findings in the nondiabetic and diabetic rats are presented in Table 3. Comparison of plasma lipid profiles indicates a

Table 2. Scores of hepatic steatosis and hepatic fibrosis staging.

Group	N (n)	Histological grades of steatosis Number of evaluated histological fields (percentage of grade of steatosis)				Fibrosis stage (N)				
		0	1	2	3	0	1	2	3	4
nDM	18 (180)	180 (100)	0 (0)	0 (0)	0 (0)	18	0	0	0	0
C-DM	6 (60)	0 (0)	3 (5)	44 (73,3)	13 (21,7)	1	4	1	0	0
IS-DM	6 (60)	0 (0)	38 (63,3)*	21 (35)*	1 (1,7)[a]	3	3	0	0	0
IS+IN-DM	6 (60)	0 (0)	29 (48,3)*	27 (45)[b]	4 (6,7)[c]	2	4	0	0	0

Steatosis data are expressed as counts and percentages in parentheses. N, number of animals; (n), number of evaluated histological fields. *P<0.0001 vs. C-DM, [a]P<0.001 vs. C-DM, [b]P<0.002 vs. C-DM [c]P<0.02 vs. C-DM.

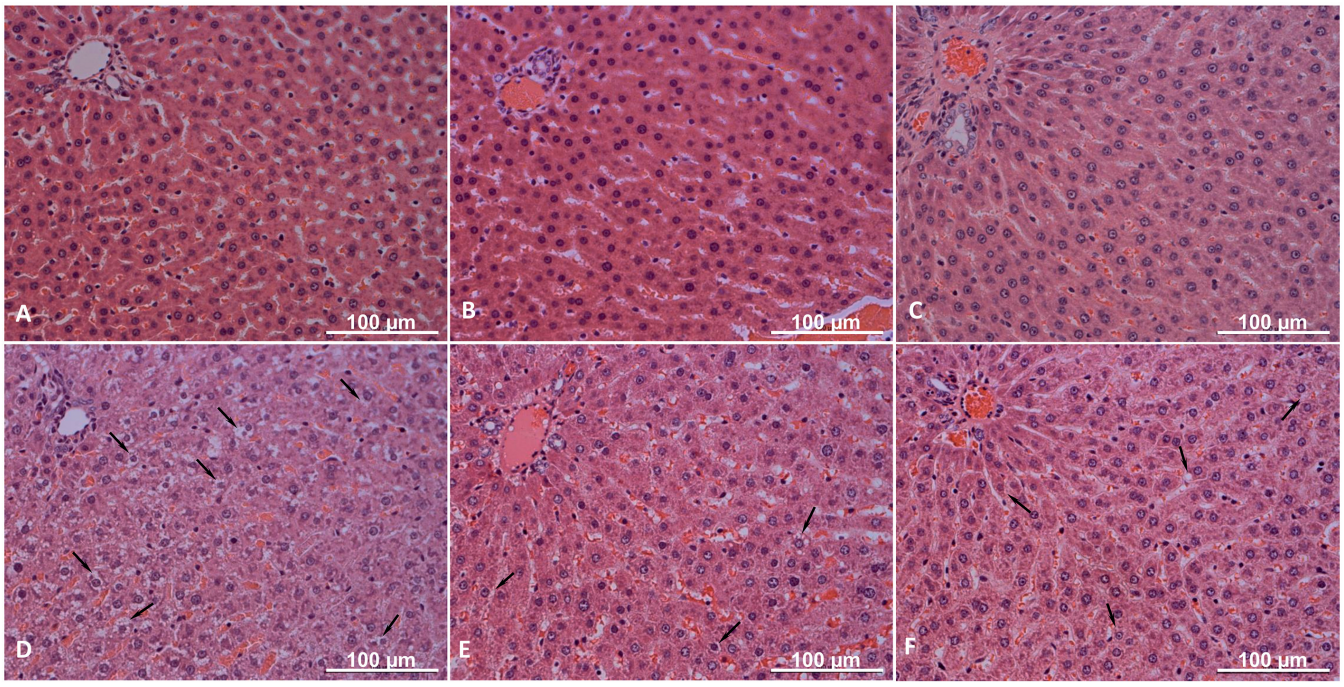

Fig. 1. Liver sections from a representative rat from each group. **A.** Control. **B.** Control supplemented with isoflavones. **C.** Control supplemented with isoflavones plus inulin. **D.** With induced diabetes mellitus. **E.** With induced diabetes mellitus supplemented with isoflavones. **F.** With induced diabetes mellitus supplemented with isoflavones plus inulin. Black arrows indicate steatosis. H-E.

statistically significant decrease of plasma HDL cholesterol in the control diabetic (C-DM) group compared to the control nondiabetic (C-nDM) group. We also noticed a statistically significant increase in plasma HDL cholesterol in the IS-DM group, as compared to the C-DM group. Comparison of the plasma lipid profiles

Table 3. Baseline plasma lipid profile.

Parameters (mg/dl)	Control group			High fat diet group		
	C-nDM	IS+nDM	IS+IN+nDM	C-DM	IS-DM	IS+IN-DM
Triglycerides	39.83±21.55	32.83±9.41	36.5±11.28	67.0±32.31	60.4±29.47	49.0±12.92
Cholesterol	59.0±7.37	56.16±6.36	56.33±3.54	51.5±8.28	70.16±19.18	51.66±10.22
HDL	19.5±1.8	20.16±3.8	20.16±3.18	16.83 ±1.46*	27.67±4.78[b]	22.66±3.54[b]
LDL	9.0±3.06	7.33±2.43	7.83±1.07	6.67±2.69	9.50±4.75	7.16±1.95

* Plasma levels of lipids from rats: C-nDM, control nondiabetic; IS-nDM, nondiabetic group supplemented with isoflavones; IS+IN-nDM, nondiabetic group supplemented with both isoflavones and inulin; C-DM, control diabetic group; IS-DM, diabetic group supplemented with isoflavones; IS+IN-DM, diabetic group supplemented with both isoflavones and inulin. Data are expressed as means±SDs of the six rats in each group. *P<0.05 vs. C-nDM; [b]P<0.05 vs. C-DM.

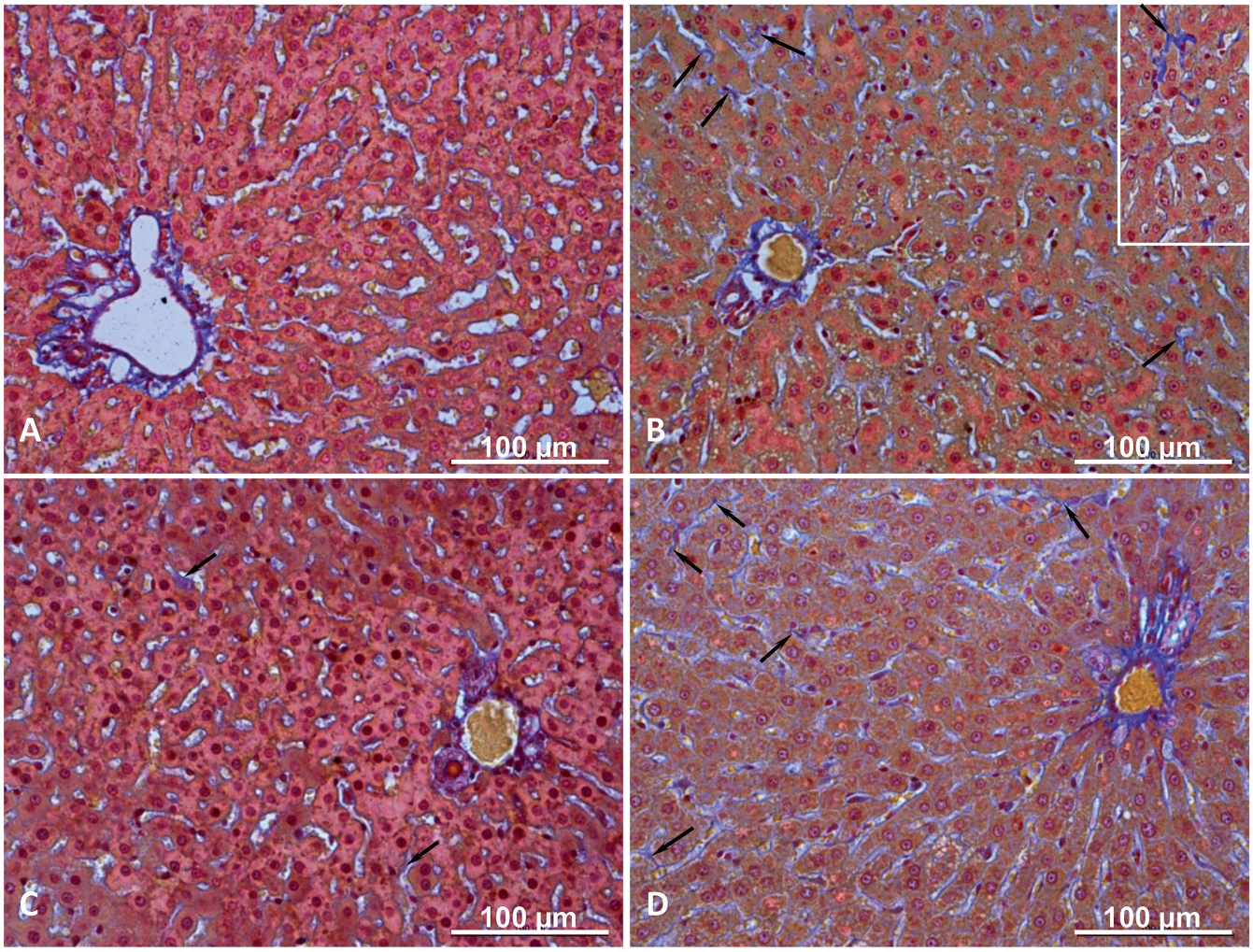

Fig. 2. Liver sections from a representative rat from each group. **A.** Non-diabetic group. **B.** With induced diabetes mellitus. **C.** With induced diabetes mellitus supplemented with isoflavones. **D.** With induced diabetes mellitus supplemented with isoflavones plus inulin. Black arrows indicate fibrotic changes. Mallory's trichrome method.

indicated a statistically significant increase in plasma HDL cholesterol in the IS+IN-DM group, as compared to the C-DM group.

Fatty acid profile

The study revealed significant changes in liver fatty

Table 4. Liver fatty acids profiles in nondiabetic and diabetic groups of rats.

Fatty acids [%]	Non-Diabetes groups			Diabetes groups		
	C-nDM	IS+nDM	IS+IN+nDM	C-DM	IS-DM	IS+IN-DM
C16:0 palmitic acid	22.81637±0.734	22.971892±0.666	24.023308±0.464*	21.28995±1.334*	22.309906±2.481	23.04933±2.314
C16:1 palmitoleic acid	0.4110703±0.103	0.3484543±0.122	0.436757±0.128	0.282331±0.145	0.3383097±0.145	0.317842±0.116
C18:0 stearic acid	18.13697±2.198	21.298259±1.738*	20.643622±1.251*	22.13488±2.721*	26.25043±3.399$^{\Delta}$	22.502054±4.47
C18:1w9 oleic acid	11.57282±2.308	9.261922±1.79^{b}	9.1312519±0.786*	15.88092±2.535**	10.000002±5.207$^{\Delta}$	12.45269±2.52$^{\Delta}$
C18:1w11 vaccenic acid	4.100382±0.211	3.4183957±0.529*	3.4531557±0.362*	2.473481±0.383**	1.8847177±0.493$^{\Delta}$	2.3867373±0.32

Data are expressed as means±SDs of the six rats in each group *P<0.05 vs. C-nDM, **P<0.01 vs. C-nDM, ^{b}P<0.055 vs. C-nDM, $^{\Delta}$P<0.05 vs. C-DM.

Table 5. SCD and DNL indices.

Parameters	Control group			High fat diet group		
	C-nDM	IS+nDM	IS+IN+nDM	C-DM	IS-DM	IS+IN-DM
SCD-16	0.018±0.005	0.015±0.006	0.018±0.006	0.013±0.007	0.015±0.007	0.013±0.004
SCD-18	0.66±0.23	0.44±0.13^{c}	0.45±0.07	0.74±0.26	0.40±0.24^{b}	0.59±0.23
DNL index	1.21±0.08	1.25±0.18	1.27±0.02	1.56±0.07^{a}	1.61±0.21	1.71±0.19

Liver fatty acids indices from nondiabetic and diabetic groups of rats. Data are expressed as means±SDs of the six rats in each group. ^{a}P<0.05 indicates statistically significant differences between DMT2 rats and CTR rats. ^{b}P<0.05 indicates statistically significant differences between DMT2+Iso and DMT2+Iso+Inu with respect to control rats. ^{c}P<0.06, which is on the border of statistical significance between IS-nDM rats and nDM rats. Data are expressed as means±SDs of the six rats in each group.

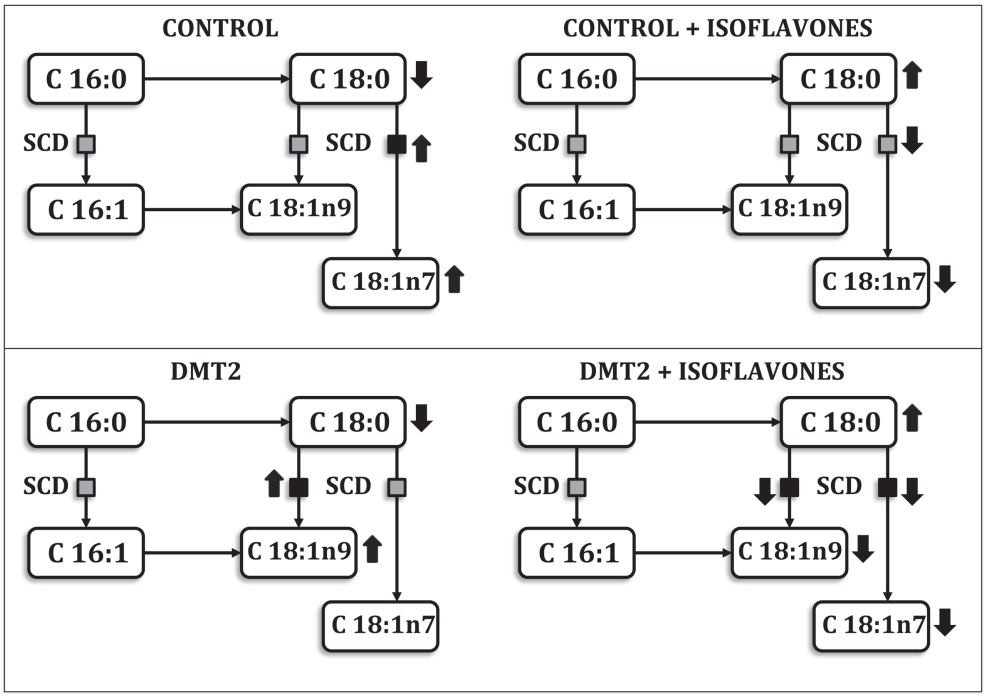

Fig. 3. Changes in SCD activity associated with isoflavone supplementation.

acids. The comparison of the control nondiabetic group with the control diabetic group showed statistically significant changes in palmitic acid, stearic acid, oleic acid, and vaccenic acid (Table 4).

Comparison of the C-nDM group with the IS-nDM group showed statistically significant changes in liver stearic acid and vaccenic acid, while oleic acid was on the border of statistical significance ($p<0.055$).

The use of isoflavones with inulin in the nondiabetic group led to statistically significant changes in palmitic acid, stearic acid, oleic acid, and vaccenic acid, as compared to the C-nDM group.

Comparison of the C-DM with the IS-DM showed statistically significant changes in stearic acid, oleic acid, and vaccenic acid, while comparison of the C-DM with the IS+IN-DM group pointed to a statistically significant change in oleic acid.

SCD index and DNL index

The SCD-18 index decreased significantly in the IS-DM group, as compared to the C-DM group. The SCD-18 index also decreased (on the border of statistical significance; $p<0.055$) in the IS-nDM group, as compared to the C-nDM group. The DNL index significantly increased in the C-DM group, as compared to the C-nDM group (Table 5).

Discussion

As expected, treatment with isoflavones had positive effects on liver morphology and plasma lipid profile in the diabetic and nondiabetic groups. Surprisingly, the positive effects of all the measured parameters (level of steatosis, fibrosis, liver SCD-1 index, liver fatty acids, and plasma lipid profile) decreased in those animals supplemented with both soya isoflavones and inulin.

Considering the plasma lipid profile, the insulin resistance found in type-2 diabetes leads to increased triglycerides and decreased concentration of HDL-C, while concentrations of LDL-C mostly did not change significantly (Chi et al., 2016). A similar tendency was observed in our experiment, which showed significantly decreased HDL-C level and increased (though not significantly) levels of triglycerides in diabetic rats, compared with nondiabetic rats. Our study showed that one month of supplementation with soy isoflavones significantly increased plasma HDL-C in that diabetic group and nonsignificantly decreased triglycerides in diabetic and nondiabetic groups. Our results were in accordance with the work of others (Taku et al., 2007; Chang et al., 2008; Shim et al., 2008; Kim et al., 2013; Chi et al., 2016). The positive effect of soya isoflavones on plasma lipid profile in both diabetic and nondiabetic individuals was decreased when isoflavones were administered together with inulin. The literature does not describe unambiguous results concerning the effects of inulin on plasma lipid profile (Venter et al., 1990; Alles et al., 1999; Bonsu and Johnson 2012). There is very little data showing the effects of both inulin and isoflavones on plasma lipid profile. A recent study indicates that 12 months of supplementation with a mixture of isoflavones, together with calcium, vitamin D, and inulin, seemed to improve the lipid profile in postmenopausal women (Vitale et al. 2018). It is possible that the effect of inulin with isoflavones depends on the duration of supplementation.

In our experiment, pharmacological induction of symptoms of type-2 diabetes through the use of low doses of STZ and a high-fat diet led to abnormalities not only in the plasma lipid profile, but also in characteristic morphological changes in the liver, with microvesicular steatosis and fibrosis. Diabetes led to liver steatosis, mainly through the excessive accumulation of fat in hepatocytes. Supplementation with soya isoflavones decreased fat content in the liver, reducing hepatic steatosis. This reduction may be associated with changes in the expression of genes involved in adipogenesis and fatty acid β-oxidation. Moreover, the decreased levels of fat in hepatocytes may also improve hepatic insulin resistance (Crespillo et al., 2011). The beneficial effect was diminished in the animals supplemented with both isoflavones and inulin.

Increased adiposity and insulin resistance can lead to the development of fibrosis. Such changes are linked with an increased number of dying hepatocytes, which promotes increased levels of reactive oxygen species and affects the production of adipokines and cytokines, which activate hepatic stellate cells (HSC) to produce excessive extracellular matrix (Chiang et al., 2011). The analysis of fibrotic changes in the liver of rats with induced diabetes revealed decreased deposition of collagen fibers after supplementation with soya isoflavones and with soya isoflavones and inulin, although the isoflavones plus inulin produced a slightly worse effect than isoflavones without prebiotics.

Hepatic *de novo* lipogenesis (DNL) is a major biosynthetic pathway in the liver, assisting in storing lipids and cell-secreted lipids in hepatocytes (Jensen-Urstad and Semenkovich, 2012). Lipogenesis is part of complex metabolic pathways in the liver and depends on glycolysis and carbohydrate metabolism. Diets rich in carbohydrates thus increases the rate of DNL. Moreover, abnormally elevated DNL is associated with the pathogenesis of nonalcoholic fatty liver disease (Donnelly et al., 2005) and with insulin resistance (Ameer et al., 2014), which is linked to the development of type-2 diabetes. The lipogenic index was significantly higher in individuals with induced type-2 diabetes than in the control group. It is no surprise that *de novo* lipogenesis functions as an adaptation for handling excess carbohydrates, which are converted to fatty acids and triacylglycerol (Chong et al., 2008). Supplementation with isoflavones or with isoflavones and inulin did not cause statistically significant changes in either the control or the diabetes group. The mechanism of DNL regulation is multifactorial and depends mainly on insulin and intracellular cholesterol level.

Another important parameter in this study was stearoyl-CoA desaturase-1 (SCD-1). SCD-1 is the key enzyme in fatty acid synthesis, responsible for introducing the first cis-double bond in the Δ9 position of stearic acid (C18:0) and palmitic acid (C16:0), both lipotoxic saturated fatty acids, to generate the less lipotoxic monounsaturated fatty acids (MUFAs) oleic acid (C18:1n-9) and palmitoleic acid (C16:1n-7), respectively (Paton and Ntambi, 2010). Lipotoxicity of SFA is common in metabolic disorders and diabetes, and is connected with the increase in intracellular fatty acid metabolites, leading to endoplasmic reticulum stress (Eizirik et al., 2008) and insulin resistance (Han and Kaufman, 2016). The overexpression of SCD may be one of the adaptive mechanisms leading to pathological changes in the liver (Pinnamaneni et al., 2006) and is connected with serious metabolic disorders (Ntambi et al., 2002; Gutierrez-Juarez et al., 2006).

The main product of hepatic SCD conversion is oleate (C18:1n9). In physiological conditions, the activity of SCD-18 (estimated through the ratio of stearic acid to oleic acid) affects membrane fluidity and cell-cell interaction (Ntambi, 1995). Changes in this ratio have been observed in several pathological states, such as diabetes, cardiovascular diseases, obesity, hypertension, neurological diseases, immune disorders, cancer, and aging (Warensji et al., 2006). Individuals with more advanced metabolic disorders are expected to have higher levels of C16:1 and C18:1. Our analysis of free fatty acids in the control diabetic rats, as compared to the control nondiabetic rats, showed that the levels of C18:0 and C18:1n-9 significantly increased, while the level of C16:0 decreased and the level of C16:1n7 did not significantly change. The addition of isoflavones decreased the activity of SCD, and particularly of SCD-18. Although oleic acid is the main diet-derived MUFA, the regulation of SCD-1 gene expression is affected by hormonal, environmental, and dietary factors, such as the consumption of high-carbohydrate food, which can rapidly induce the SCD-1 gene through insulin-mediated increases in the activation of sterol regulatory element-binding protein (SREBP)-1c and the SCD-1 gene promoter (Paton and Ntambi, 2008). Kotronen et al. demonstrated that SCD-1 activity increases with increasing hepatic fat content in the human liver (Kotronen et al., 2009). Moreover, experiments on mice with knockout SCD-1 gene revealed decreased adiposity due to reduced lipid synthesis and increased energy outlay through elevated fatty acid oxidation, which is associated with protection against obesity (Miyaki et al., 2000; Ntambi et al., 2002). The loss of the SCD-1 gene increases insulin and glucose uptake in muscle and brown adipose tissue (Rahman et al., 2005). The changes in SCD indices associated with isoflavone supplementation are shown in Figure 3.

In our experiment, the SCD-18 index was significantly lower in isoflavone diabetic and isoflavone nondiabetic animals than in the control diabetic group and control nondiabetic group, respectively. Soy isoflavones show estrogenic activity by binding to estrogen receptors (ERs). ERs activated by ligands regulate a wide range of gene expression, including the expression of genes involved in lipid metabolism. Experiments on animals with estrogen deficiency (such as ovariectomized mice and estrogen receptor knockout mice) have revealed increased expression of lipogenic genes in liver and adipose tissue caused by increased expression of SCD-1 (Paquette et al., 2008). In the present study, the SCD-18 index decreased while the SCD-16 index did not change after isoflavone supplementation in both diabetic and nondiabetic animals. This seems to be in agreement with the literature (Warensji et al., 2009). The results may also vary depending on the examined tissue.

The use of both isoflavones and inulin did not lead to any significant changes in SCD-1 indices in either the control or diabetic groups in our study. These results are contrary to expectations (Piazza et al., 2007). On the other hand, Palafox-Carlos et al. (2011) suggested that the presence of high amounts of fiber can limit the bioavailability of antioxidants present in food. Antioxidants may interact with fiber macromolecules, forming chemical complexes and colloidal structures that reduce or improve their bioavailability (Palafox-Carlos et al., 2011). Moreover, the addition of inulin may have different effects through the action of microbiota and the increased extraction of energy from the diet (Turnbaugh et al., 2008). Intestinal microbiota can break down indigestible dietary fibers, producing short-chain fatty acids-mostly acetate, propionate, and butyrate. These end products provide energy and act as metabolic regulators. Acetate can be converted to acetyl-CoA, and so may be associated with lipogenesis and cholesterol synthesis (Zambell et al., 2003). Propionate has an adverse effect, inhibiting the uptake of acetate and decreasing fatty acid synthesis in hepatocytes (Demigne et al., 1995). The end products of different dietary fibers may be different and may occur in different quantities, so the metabolic effects and energy metabolism may also be different.

Conclusion

In conclusion, supplementation with 100 mg of soya isoflavones for one month exerted favorable effects on plasma HDL-C and liver SCD-18 index in rats with induced type-2 diabetes. Moreover, supplementation with soya isoflavones diminished the level of steatosis and fibrosis in the liver of rats with induced type-2 diabetes. Additional supplementation with inulin decreased the positive effect of soya isoflavones, which may be associated with increased energy extraction from the diet (the addition of inulin may have different effects through the action of microbiota), interaction with fiber molecules, the duration of supplementation, or other factors. Further studies with different dosages of inulin and isoflavones and durations of supplementation should therefore be performed.

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